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Note

Determination of pelanserin in human plasma by high-performance liquid chromatography with amperometric detection

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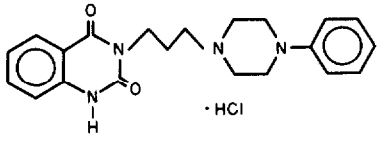
Pelanserin (Fig. 1), a quinazolinedione derivative, is an effective antihypertensive agent [1]. We have previously reported a procedure for the quantitation of pelanserin plasma concentrations by high-performance liquid chromatography (HPLC) with UV detection that requires 2-ml samples. This



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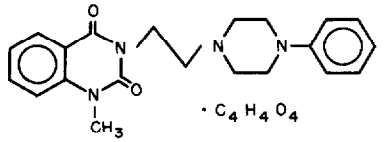

II

Fig. 1. Structures of pelanserin (I) and the internal standard (II).

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method has a detection limit of 3 ng/ml (signal-to-noise ratio = 3) and it has proved to be suitable for the determination of pelanserin pharmacokinetics in humans after oral doses of 20 mg [2]. However, recent clinical trials have shown that pelanserin produces a significant antihypertensive effect at an oral dose of 5 mg, with minimal occurrence of side-effects [3]. At this dose, pelanserin plasma levels are frequently lower than the above-mentioned limit of detection.

Electrochemical detection has recently been widely used for the analysis of easily oxidizable or reducible compounds, owing to its high selectivity and sensitivity [4]. Moreover, it has been reported that pelanserin yields a high-current response when assayed by this method [5].

This paper describes a procedure for the detection of pelanserin in plasma samples, using HPLC coupled with amperometric detection, with a detection limit of 0.3 ng/ml (signal-to-noise ratio = 3).

EXPERIMENTAL

Chemicals and solutions

Pelanserin and the internal standard, 2,4-(1*H*,3*H*)-quinazolinedione-1-methyl-3-[2-(4-phenyl-1-piperazinyl)ethyl] maleate (Fig. 1), were obtained from the Sección de Terapéutica Experimental, Cinvestav-IPN (Mexico City, Mexico). Acetonitrile, chromatographic grade, was purchased from E. Merck (Darmstadt, F.R.G.). Deionized water was prepared using a Milli-Q reagent water system (Continental Water Systems, El Paso, TX, U.S.A.). All other reagents were of analytical grade.

Pelanserin stock solutions were prepared in deionized water at a concentration of 0.912 mg/ml (free base). Internal standard stock solutions were prepared in methanol at a concentration of 0.760 mg/ml (free base). Standard solutions were prepared by serial dilutions from stock solutions with deionized water. Pelanserin and internal standard concentrations are always referred to the free base.

The following aqueous solutions were prepared: 0.1 *M* sodium acetate solution, pH adjusted to 4.0 with glacial acetic acid; 0.025 *M* sodium borate (pH 9.0) and 0.1 *M* hydrochloric acid, all prepared in deionized water.

Sample preparation

Blood was collected from healthy subjects before and after pelanserin oral administration. Samples were drawn by puncture of a suitable forearm vein and blood collection into heparinized vacutainers. Plasma was obtained by centrifugation at 3000 *g* for 15 min. A 1-ml plasma specimen (unknown samples, drug-free plasma or plasma standards containing known pelanserin amounts) was pipetted into a conical glass tube (tube A) and spiked with 30.4 ng (0.1 ml of a 304 ng/ml solution) of internal standard. After addition of 1

ml of sodium borate solution, plasma was extracted with 4 ml of diethyl ether using a Yankee variable-speed rotator (Clay Adams, Parsippany, NJ, U.S.A.) set at 180 rpm. The two phases were separated by centrifugation at 3000 *g* for 5 min. The upper organic layer was transferred to another conical glass tube (tube B), and the solvent was evaporated to dryness at 45°C under a gentle stream of nitrogen. The plasma was extracted again with 2 ml of diethyl ether, and the organic layer was transferred to tube B, dissolving the dry residue from the previous extraction. Then a back-extraction step was performed with 0.5 ml of 0.1 *M* hydrochloric acid, and 0.2-ml aliquots of the acid layer were injected into the chromatographic system.

Chromatographic conditions

Extracted plasma samples were separated by reversed-phase HPLC. Detection was carried out by measuring the current response produced by the column effluent in an electrochemical cell maintained at a fixed voltage.

The chromatographic system consisted of a Model 510 solvent-delivery system (Waters Assoc., Milford, MA, U.S.A.), a Model LC-22A temperature controller (BAS, West Lafayette, IN, U.S.A.), an electrochemical transducer coupled to a Model LC-4B amperometric detector (BAS) and a 200-μl loop injector (Rheodyne, Cotati, CA, U.S.A.). Analyses were performed on a 300 mm$\times$4 mm I.D. MCH-10 column of 10 μm particle size (Varian, Palo Alto, CA, U.S.A.) with acetonitrile–0.1 *M* acetate buffer (pH 4) (28:72, v/v) containing 75 mg/l EDTA as the mobile phase. The column was kept at 40°C and flow-rate was kept constant at 1.8 ml/min.

As there was no information available concerning the electrochemical properties of pelanserin, the peak current was recorded at several applied potentials and the hydrodynamic voltammogram was constructed (Fig. 2). It appeared that +850 mV was the most suitable potential as a high-current response was observed maintaining a high selectivity. Current was detected at a sensitivity of 2 or 5 nA full scale, depending on the amount of pelanserin injected into the system. To prolong the life of the analytical column, a precolumn (35 mm$\times$4 mm I.D.) containing 37–50 μm particle size Corasil C_{18} (Waters Assoc.) was used.

Calibration

The assay was calibrated by addition of known amounts of pelanserin and of the internal standard to drug-free plasma samples (1 ml). Calibration graphs were established for pelanserin free base concentrations ranging from 0.912 to 45.6 ng/ml. The internal standard was used at a fixed free base concentration of 30.4 ng/ml. The ultimate plasma concentrations were calculated by determination of the peak-height ratios of pelanserin to the internal standard.

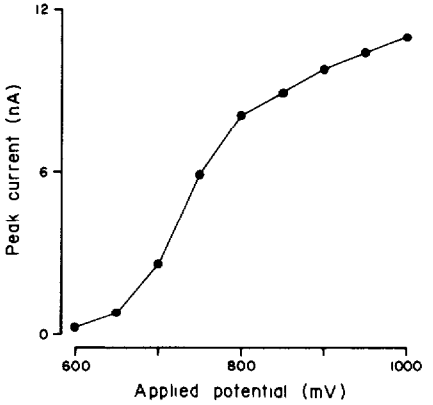

Fig. 2. Hydrodynamic voltammogram of pelanserin. Output current was determined by injecting samples of the same concentration (50 ng in 0.2 ml) at various potentials.

RESULTS

Typical chromatograms of extracted plasma samples are shown in Fig. 3. The retention times for pelanserin and the internal standard were 7.4 and 12.8 min, respectively. No interfering peaks occurred at these times. The peak at 7.4 min was identified as pelanserin by analysis of samples using parallel dual-electrode detection [6]. In this detection configuration two glassy carbon electrodes are placed side by side in the flow stream, allowing simultaneous detection of the current response at two different applied potentials. Measurements were carried out at +850 and +800 mV. Injection of pelanserin standards yielded a +850 mV/+800 mV response ratio of 1.4. Similar results were observed with extracted drug-free plasma samples spiked with pelanserin. Plasma samples drawn from healthy volunteers 4 h after oral administration of 20-mg pelanserin hydrochloride capsules also yielded a +850 mV/+800 mV responses ratio of 1.4.

Any endogenous contaminants remaining in the extracts were eluted before pelanserin, and samples could be injected immediately after elution of the internal standard. A linear relationship ($r=0.9998$) was found when the ratio of the peak height of pelanserin to that of the internal standard was plotted on the y axis against various concentrations of pelanserin (free base) ranging from 0.912 to 45.6 ng/ml on the x axis. The equation obtained by the least-squares method was $y=0.0899x-0.0121$.

Recoveries for pelanserin and for the internal standard, assessed by comparison of peak heights from plasma extracts with those from standard solutions, were similar and ranged from 85 to 95%. Intra-assay accuracy and precision were determined by adding known amounts of pelanserin to drug-free

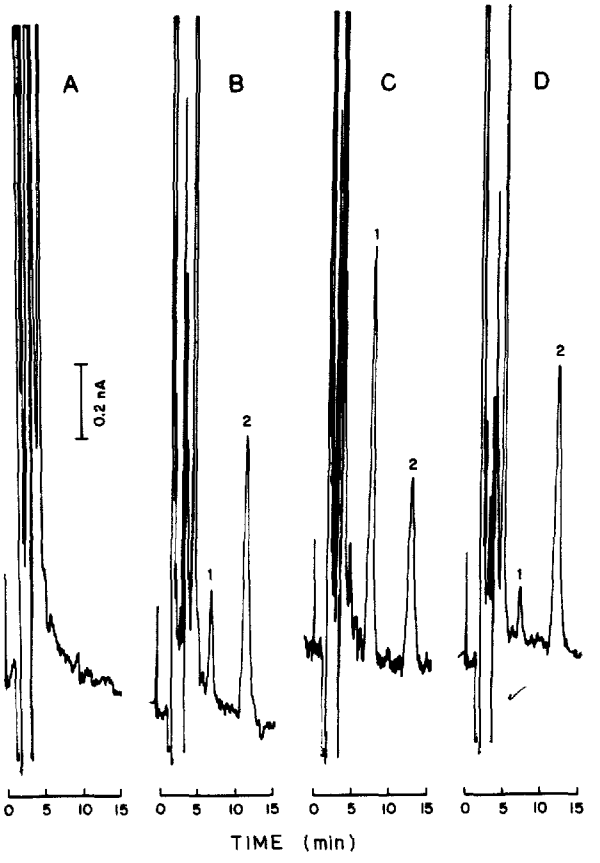

Fig. 3. Chromatograms of human plasma samples. (A) Drug-free plasma. (B) Spiked plasma containing 4.56 ng/ml pelanserin and 30.4 ng/ml internal standard. (C and D) Plasma samples taken 3 h after the first dose (C) and 12 h after the 14th dose (D) from a subject during chronic treatment with capsules containing 5 mg of pelanserin hydrochloride b.i.d. spiked with 30.4 ng/ml internal standard. Peaks: 1 = pelanserin; 2 = internal standard.

plasma and analysing the samples. The results are shown in Table I. Day-to-day precision of the assay was determined over a period of four weeks ($n=6$), coefficients of variation (C.V.) being 8.4% at 1.824 ng/ml, 5% at 9.12 ng/ml and 5.4% at 45.6 ng/ml. The detection limit (signal-to-noise ratio = 3) was 0.3 ng/ml. To examine the stability of pelanserin in plasma, drug-free plasma specimens were spiked with known drug amounts, extracted, kept frozen at $-20°C$ for different time intervals and then analysed. It was found that pelanserin plasma samples stored under these conditions are stable for at least six months.

The application of the method in clinical situations was evaluated. Four healthy volunteers were treated with 5-mg pelanserin hydrochloride capsules

TABLE I

INTRA-ASSAY ACCURACY AND PRECISION OF THE HPLC METHOD FOR DETERMINATION OF PELANSERIN IN HUMAN PLASMA SAMPLES

Theoretical concentration (ng/ml)	Observed concentration (mean ± S.E.M., $n=6$) (ng/ml)	Accuracy (%)	Coefficient of variation (%)
0.912	0.962 ± 0.024	105.4	6.1
1.824	1.760 ± 0.034	96.5	4.8
4.56	4.564 ± 0.129	100.1	6.9
9.12	8.962 ± 0.183	98.3	5.0
45.6	45.632 ± 0.671	100.0	3.6

TABLE II

PLASMA LEVELS OF PELANSERIN DURING CHRONIC ORAL TREATMENT

Four healthy male volunteers were given capsules containing 5 mg of pelanserin hydrochloride b.i.d.

Day of treatment	Concentration (mean ± standard error of the mean) (ng/ml)	
	0 h	3 h
1	0 ± 0	19.51 ± 2.76
8	2.60 ± 0.44	22.02 ± 4.88
15	2.90 ± 0.31	21.24 ± 4.55
22	2.54 ± 0.53	27.06 ± 6.92
29	2.69 ± 0.44	20.14 ± 3.53

b.i.d. for four weeks. Blood samples were drawn immediately before and 3 h after the morning pelanserin administration on the 1st, 8th, 15th, 22nd and 29th days of treatment. Pelanserin plasma levels were determined by the procedure described. Results are shown in Table II.

DISCUSSION

It is clear that evaluation of pharmacokinetic data and monitoring of plasma concentrations contribute to a better understanding of therapeutic efficacy and lead to a rational basis for the establishment of a proper dosage regimen [7]. We have previously reported a method for the quantitation of pelanserin in plasma by HPLC with UV detection [2]. Although this method is suitable for pharmacokinetic studies after oral dosing with 20 mg of pelanserin hydrochloride, its detection limit (3 ng/ml) does not allow pelanserin kinetics to be followed completely after 5-mg dosing. As can be seen in Table II, pelanserin

plasma levels observed 12 h after a 5-mg oral administration are frequently less than 3 ng/ml. Therefore, we designed the quantitation procedure described here, using HPLC with amperometric detection. This method has two main advantages over the previous one: it uses 1 ml instead of 2 ml of plasma, and the detection limit is ten times lower. This is achieved because pelanserin yields a high current response in amperometric detection, a procedure that is also highly selective [4].

The identity of the pelanserin peak (7.4 min) was confirmed by amperometric detection using the parallel dual-electrode mode. This configuration is analogous to dual-wavelength UV detection and has been shown to be useful for peak identification and for confirmation of peak purity [6,8]. The fact that the current response ratio +850 mV/+800 mV was the same for pelanserin standards, for spiked drug-free plasma samples and for samples drawn from subjects 4 h after oral pelanserin dosing (1.4 in all cases) suggests peak homogeneity at 7.4 min, corresponding to pure pelanserin. Thus, coelution of eventual metabolites not chromatographically separated from the parent drug seems unlikely.

The +850 mV/+800 mV current response ratio observed with the parallel dual-electrode mode was 1.4, which is slightly higher than the value of 1.2 calculated from the hydrodynamic voltammogram shown in Fig. 2. This difference is due to electrode passivation that produces day-to-day variation in electrode responsiveness. However, this variation does not influence pelanserin quantitation, as long as the system is calibrated daily [6]. Following this practice, we observed a day-to-day C.V. of less than 10%.

The present method has been shown to be adequate for clinical and pharmacokinetic studies of pelanserin after administration of doses in the therapeutically relevant range.

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